# Development of Protein Polycrystallography

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**Introduction – powders?**
**Real time & radiation damage studies**
***Ab initio*??**

**References:**

***Modern Powder Diffraction*, Eds. J. Post & D. Bish**

***The Rietveld Method*, Ed. R.A. Young**

**Von Dreele, *J. Appl. Cryst.* 32, 1084 (1999),**

**Von Dreele, *et al., Acta Cryst.* D56, 1549-1553 (2000),**

**Von Dreele, *Acta Cryst*. D57, 1836-1842 (2001)**

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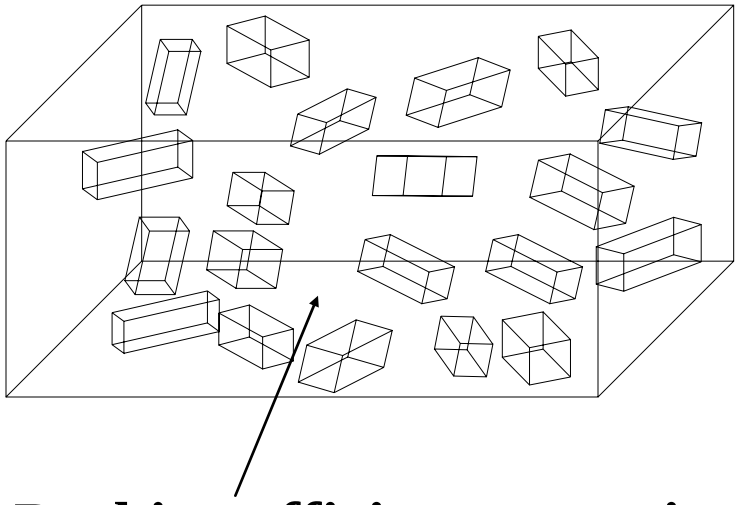
# What is a powder? - polycrystalline mass



**All orientations of crystallites possible**

**Sample: 1μl powder of 1μm crystallites - ~$10^9$ particles**

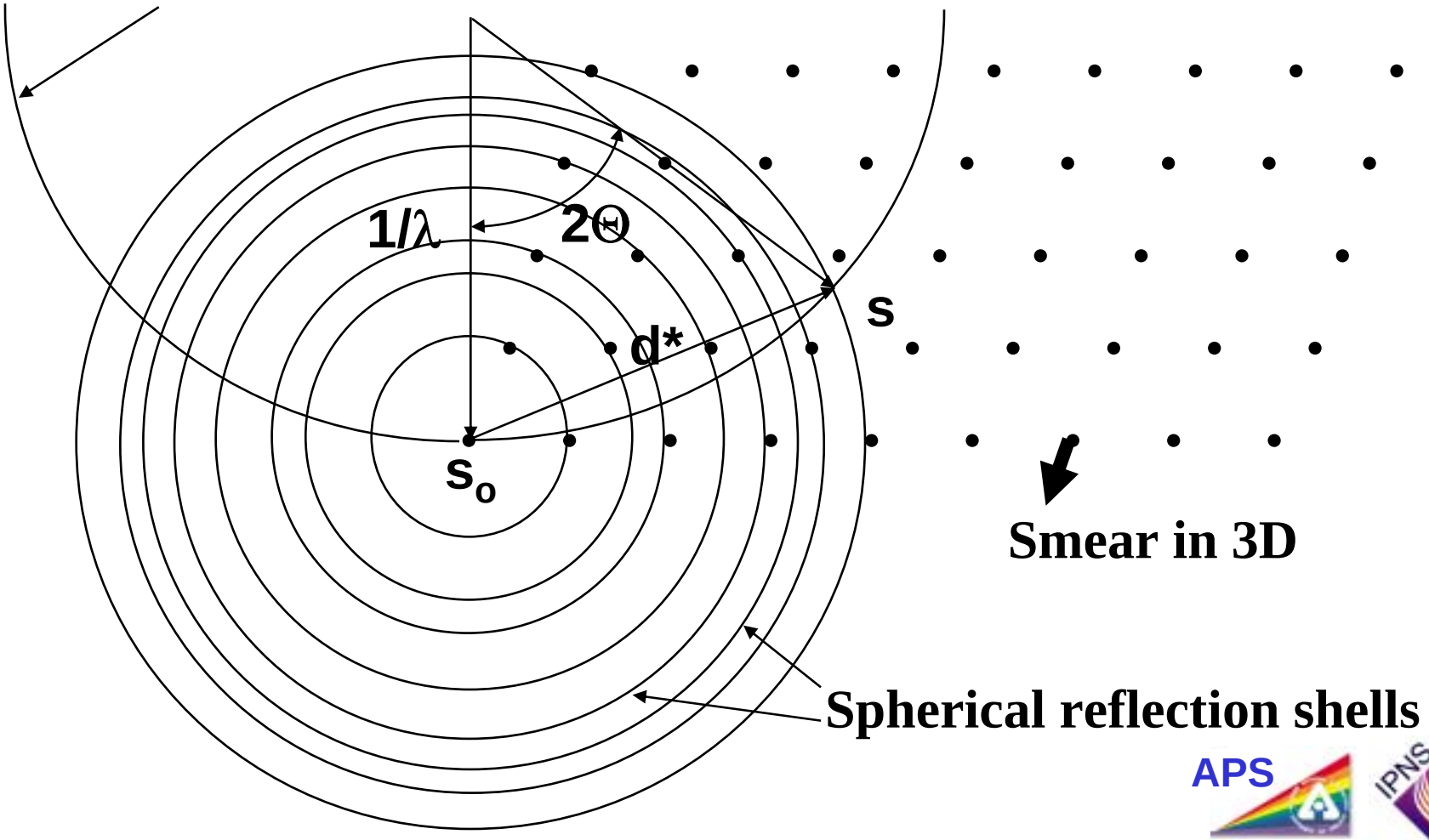
**Packing efficiency – typically 50%**
**Spaces – air, solvent, etc.**

**Single crystal reciprocal lattice**
**- smeared into spherical shells**

# Powder diffraction - reciprocal space

**Bragg's Law** $d^*/2 = \sin\Theta/\lambda$

**Ewald sphere**

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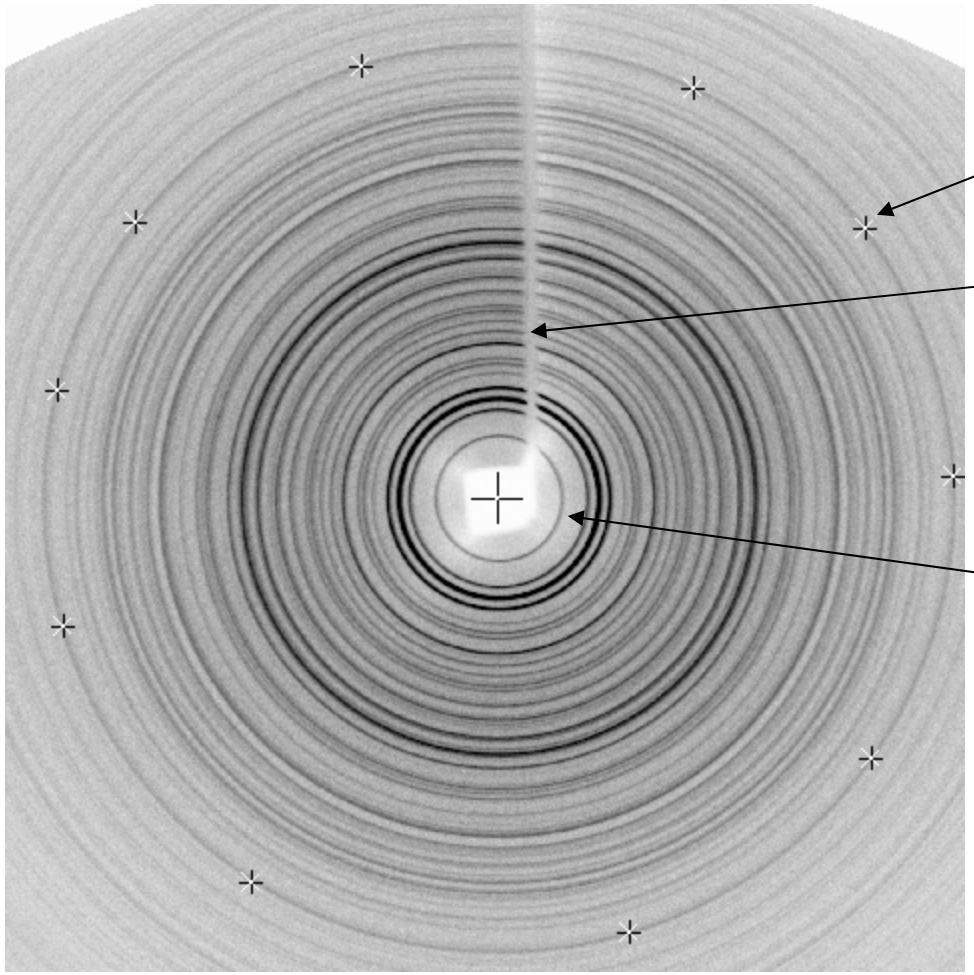
# Rings – protein pattern (HEWL)



Centering ring @
4.6deg 2Θ

Beam stop holder

Inner most ring – d~55Å
(110) Reflection, lowest order
for tetragonal lysozyme
2Θ ~ 0.67deg

Texture free sample
& no graininess

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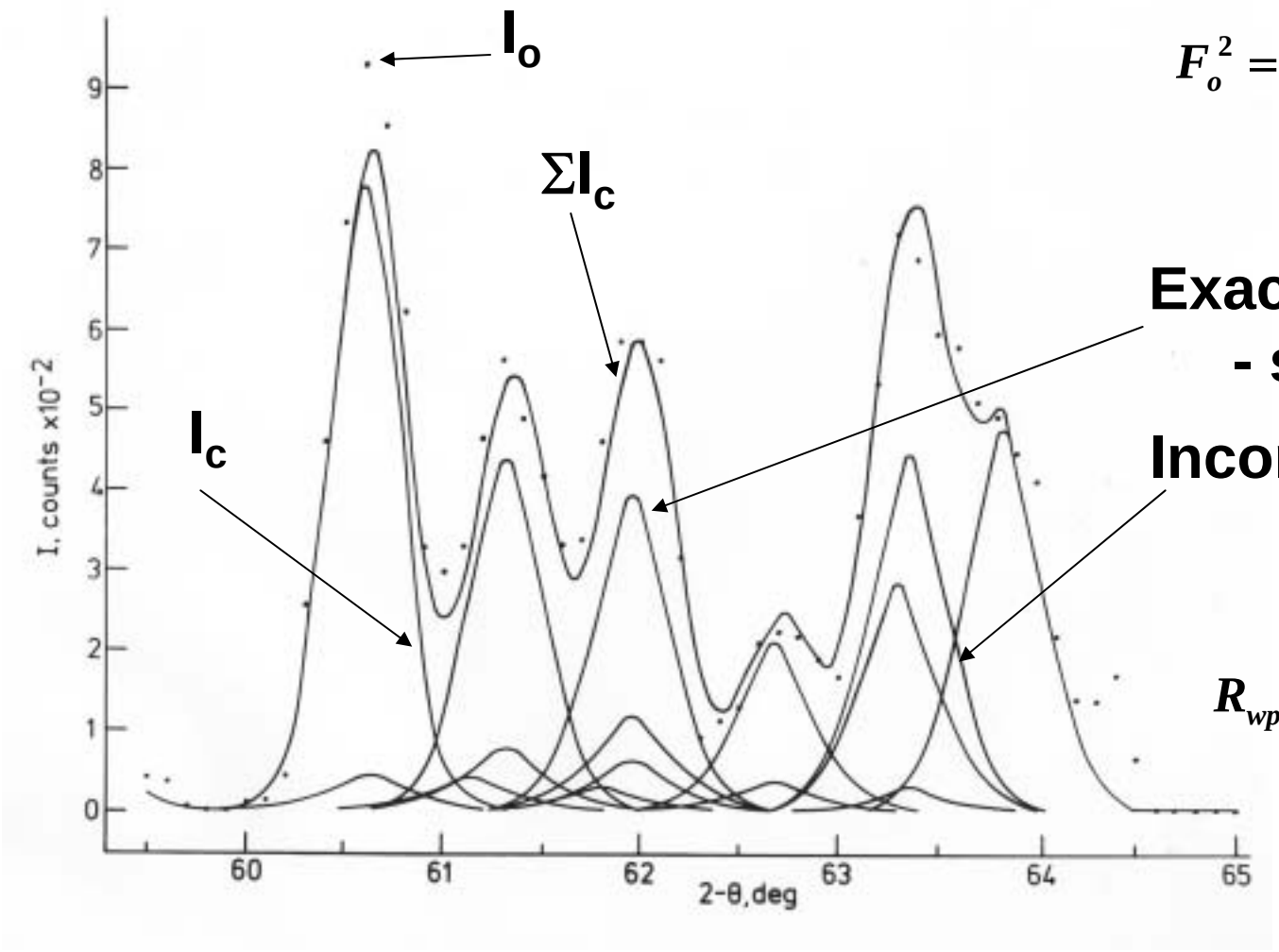
# Rietveld refinement – extract structure factors

## Rietveld Minimize

$$M_R = \sum w(I_o - I_c)^2$$

## Apportion $I_o$ by ratio of $I_c$ to $\Sigma I_c$ & apply corrections

$$F_o^2 = \frac{1}{Lp} \sum I_o \left( \frac{I_c}{\sum I_c} \right)$$




**Exact overlaps - symmetry**

**Incomplete overlaps**

**Residuals:**

$$R_{wp} = \sqrt{\frac{\sum w(I_o - I_c)^2}{\sum w I_o^2}}$$

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# Stereochemically restrained Rietveld refinement - minimization function (typical protein technique)

$$M = f_y \sum w_i \left(Y_{oi} - Y_{ci}\right)^2$$ **Powder profile (Rietveld)**

$$+ f_a \sum w_i \left(a_{oi} - a_{ci}\right)^2$$ **Bond angles**

$$+ f_d \sum w_i \left(d_{oi} - d_{ci}\right)^2$$ **Bond distances**

$$+ f_t \sum w_i \left(-T_{ci}\right)^2$$ **Torsion angle pseudopotential**

$$+ f_p \sum w_i \left(-p_{ci}\right)^2$$ **Plane RMS displacements**

$$+ f_v \sum w_i \left(v_{oi} - v_{ci}\right)^4$$ **van der Waals distances**

$$+ f_h \sum w_i \left(h_{oi} - h_{ci}\right)^2$$ **Hydrogen bonds**

$$+ f_x \sum w_i \left(x_{oi} - x_{ci}\right)^2$$ **Chiral volumes**

$$+ f_R \sum w_i \left(-R_{ci}\right)^4$$ **"$\phi/\psi$ or $\chi_1/\chi_2$" pseudopotential**

**$w_i = 1/\sigma^2$ weighting factor**

**$f_x$ - weight multipliers (typically 0.1-10)**

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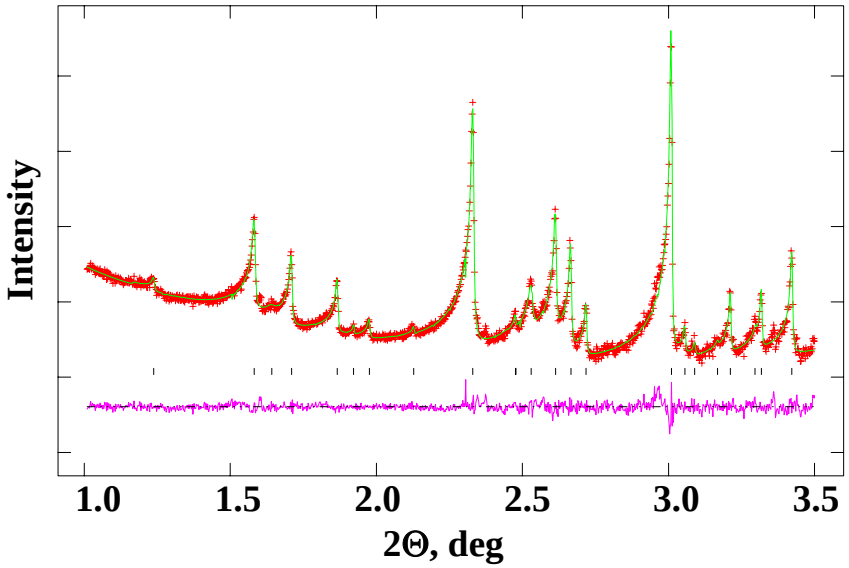
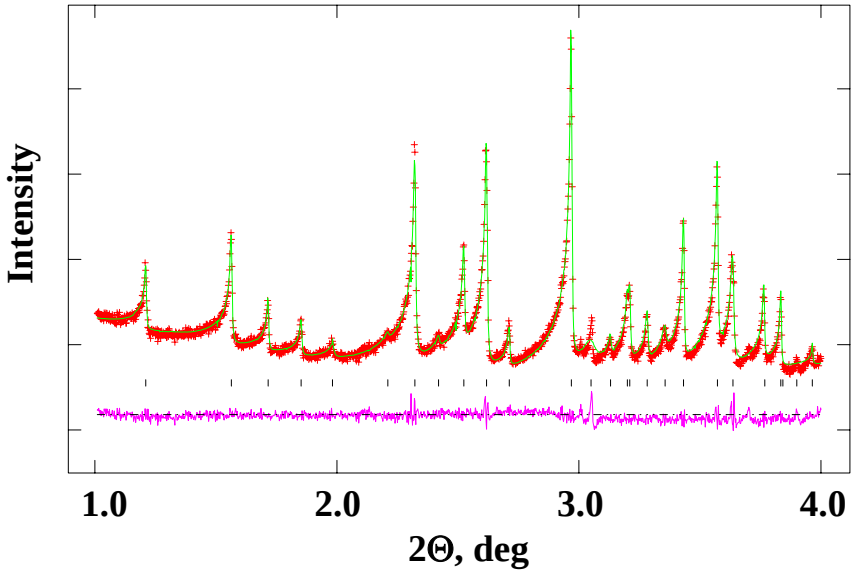
# $T_3R_3$ Zn insulin – phase change by grinding

**Grind $T_3R_3$ complex in agate mortar with mother liquor**
**High resolution synchrotron x-ray powder patterns (X3b1/NSLS)**




**Immediately after grinding**
**Indexed – R3**
**a=81.275Å, c=73.024Å**
**New phase – $T_3R_3DC$**
**Solve by molec. repl.**

**After 2 days rest**
**Indexed – R3**
**a=81.084Å, c=37.537Å**
**same as single xtal**

**Doubled cell**

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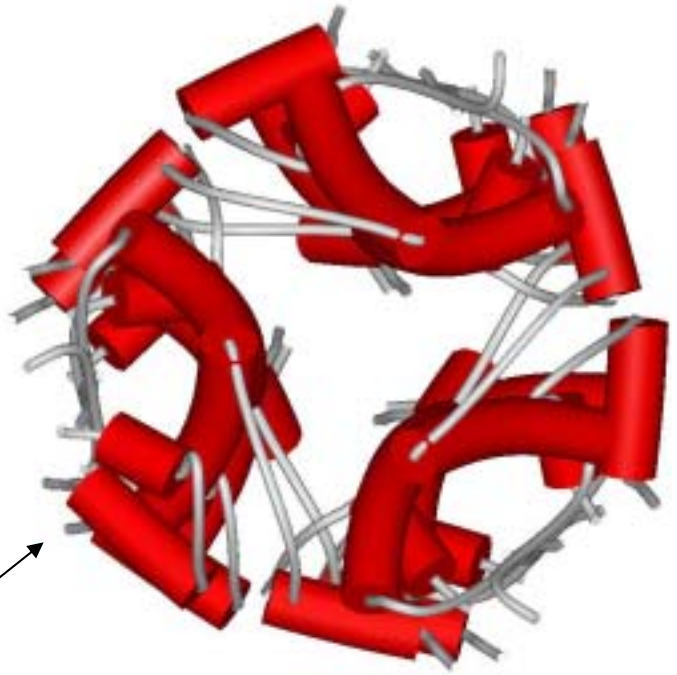
# Schematic of $T_3R_3DC$ Zn-insulin complex.

**Powder RT structure PDB=1FUB**

**View down 3-fold axis**
**Front $T_3R_3$ turned 9$^o$**
**wrt back $T_3R_3$**



**Same structure as --**
**Single crystal – Lo T phase PDB=1G7A**

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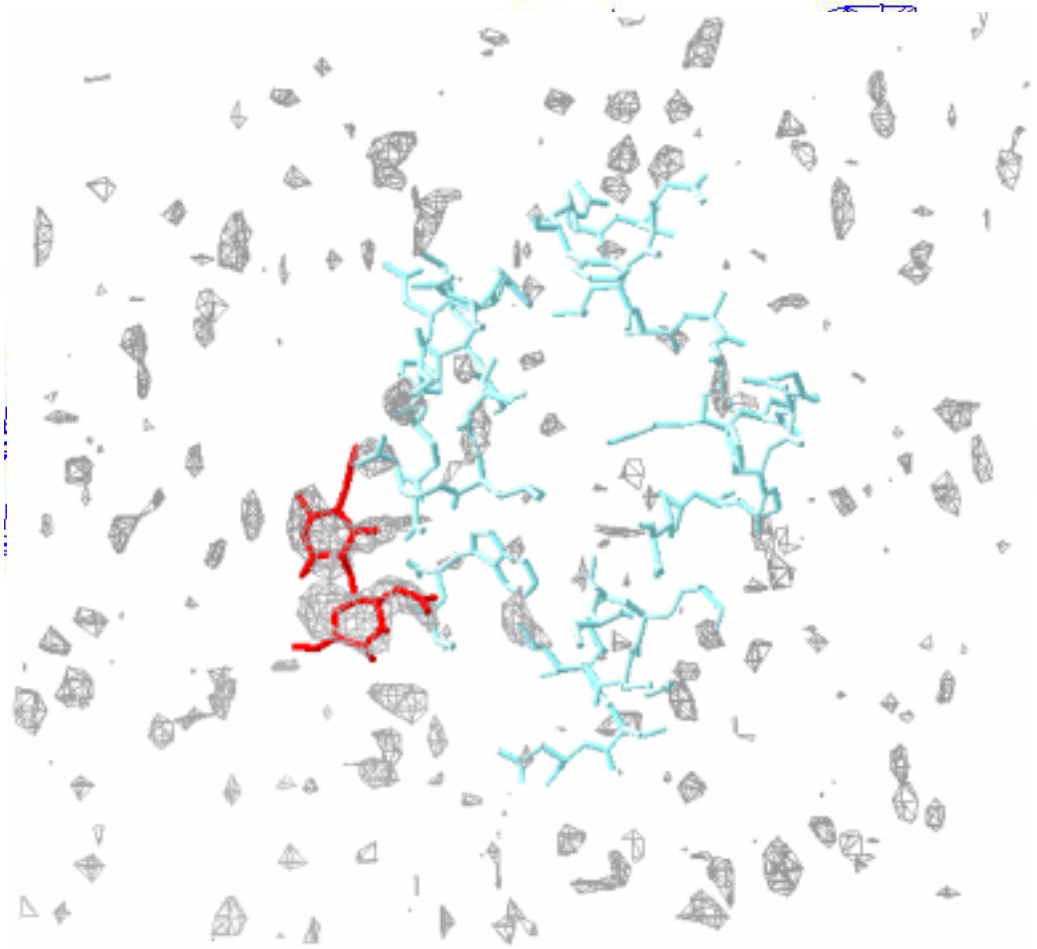
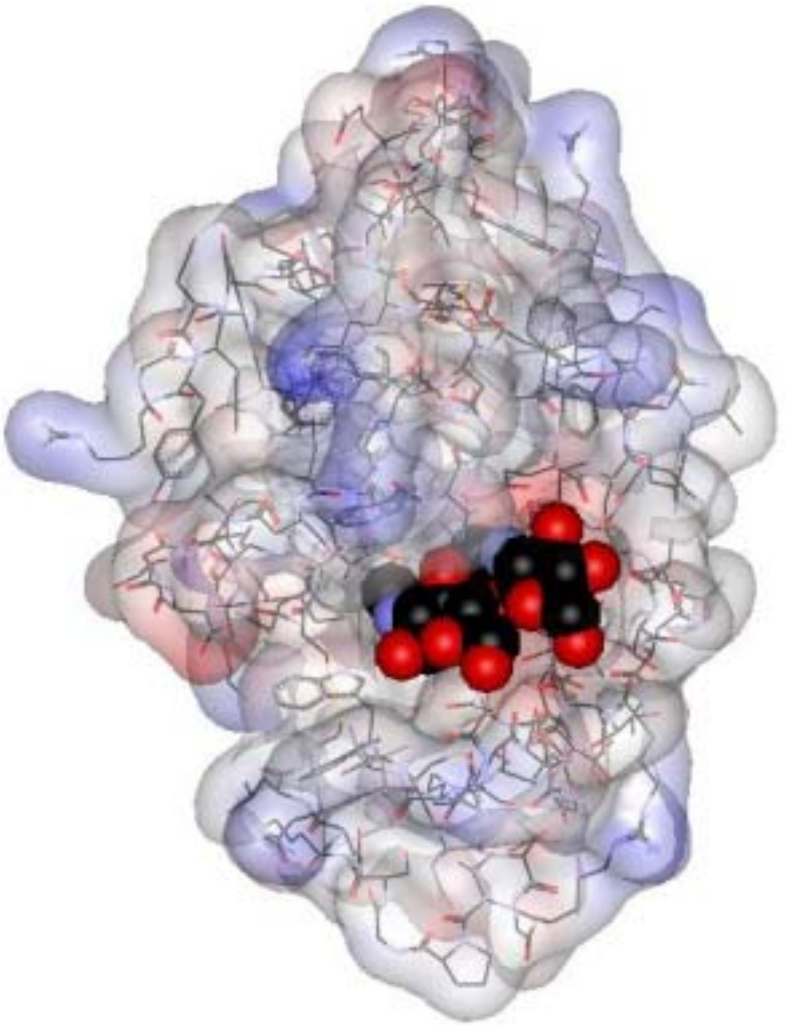
# LYSO/$NAG_2$ – powder data from complex





**ΔF map from HEWL & extracted $F_o$**

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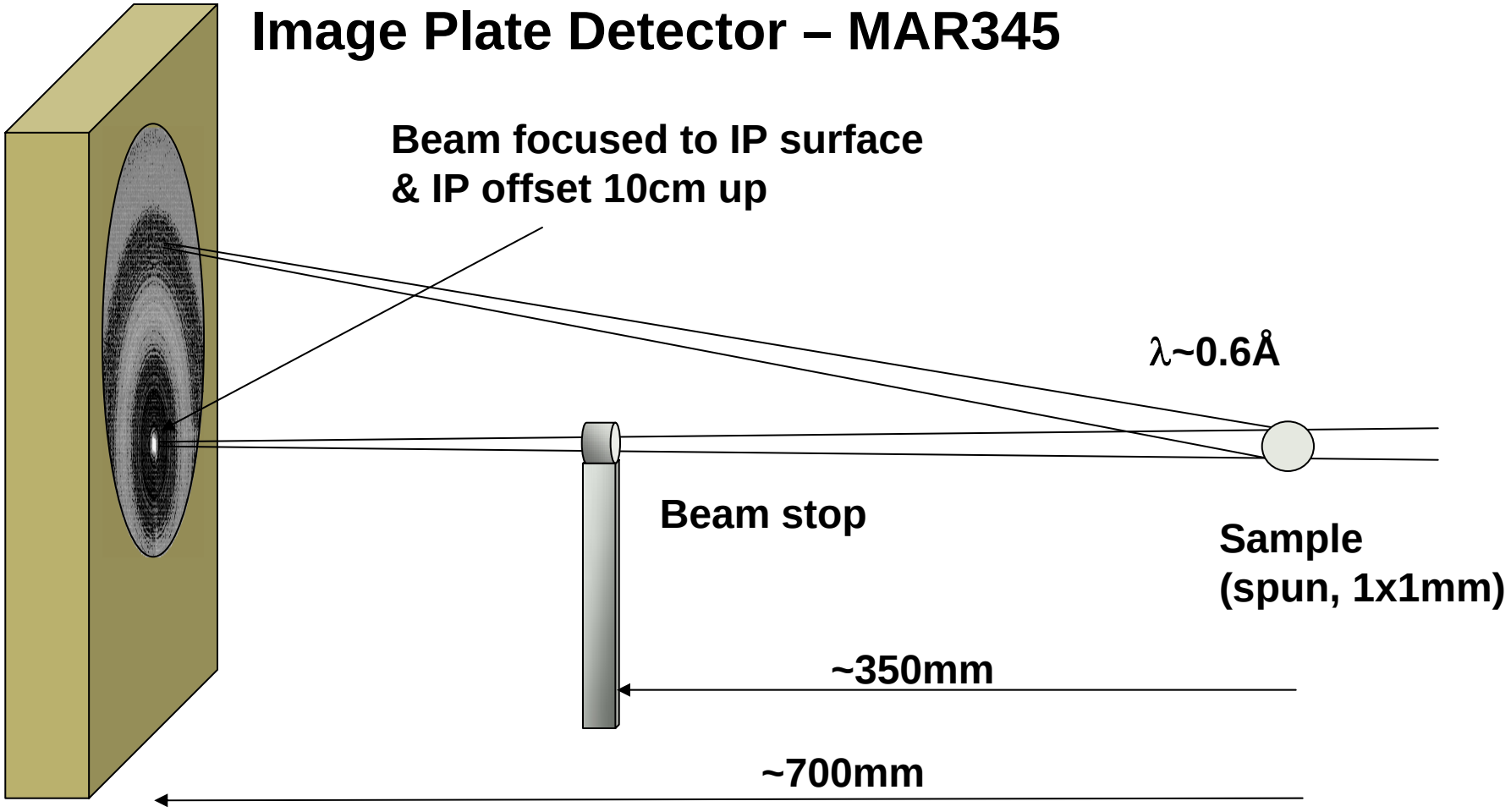
# Better data collection

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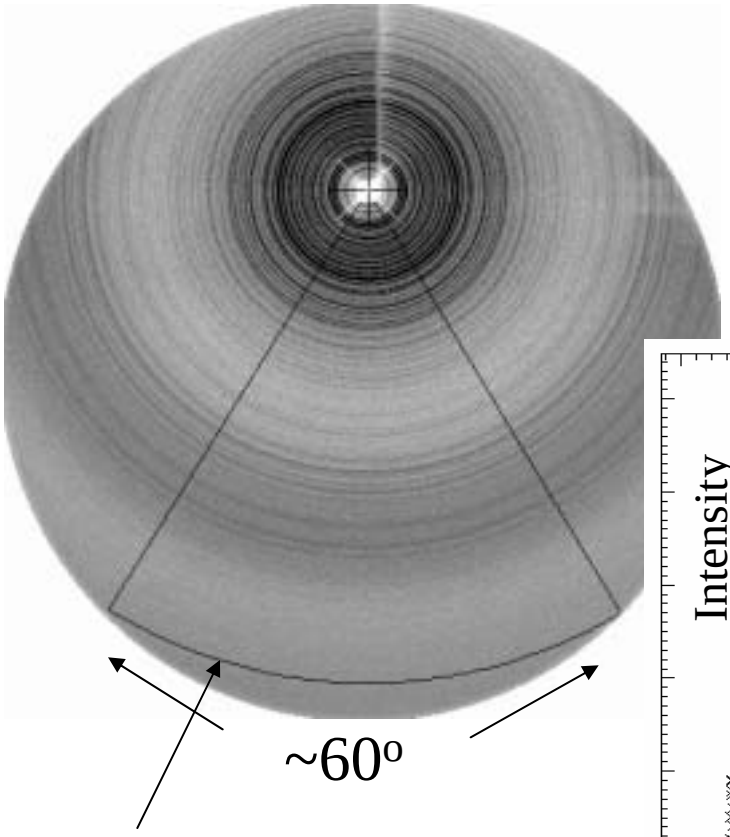
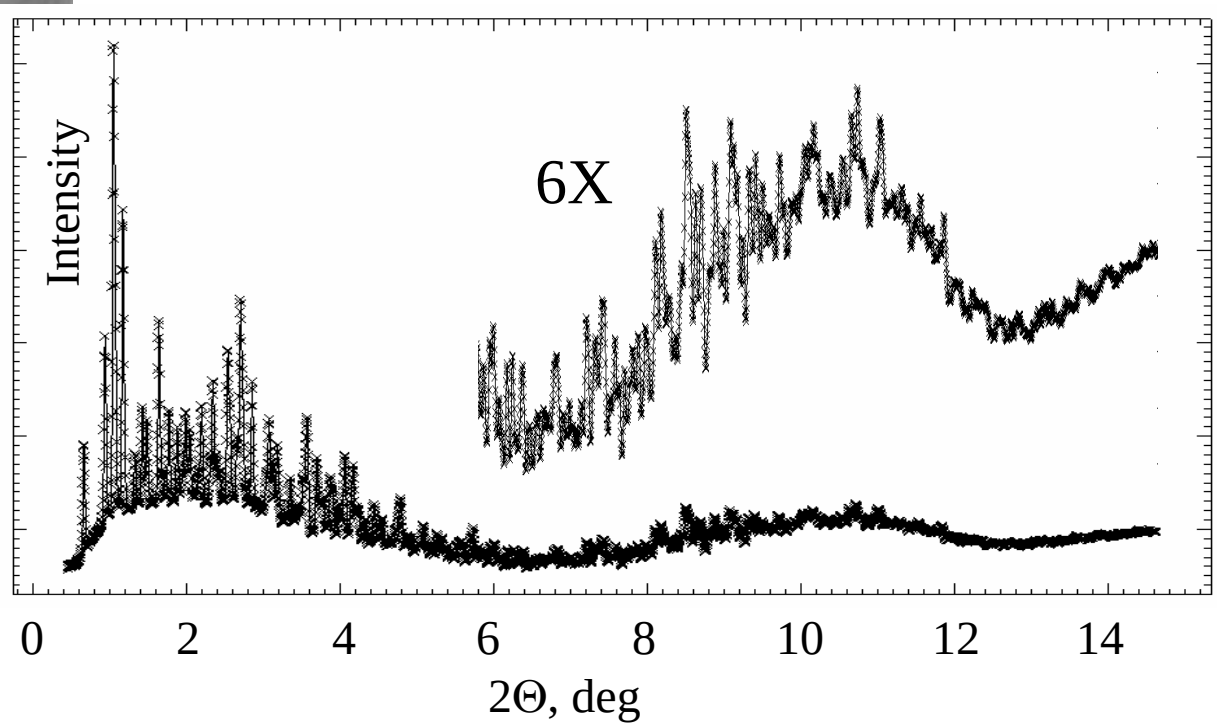
# Integration – fit2d “cake” option



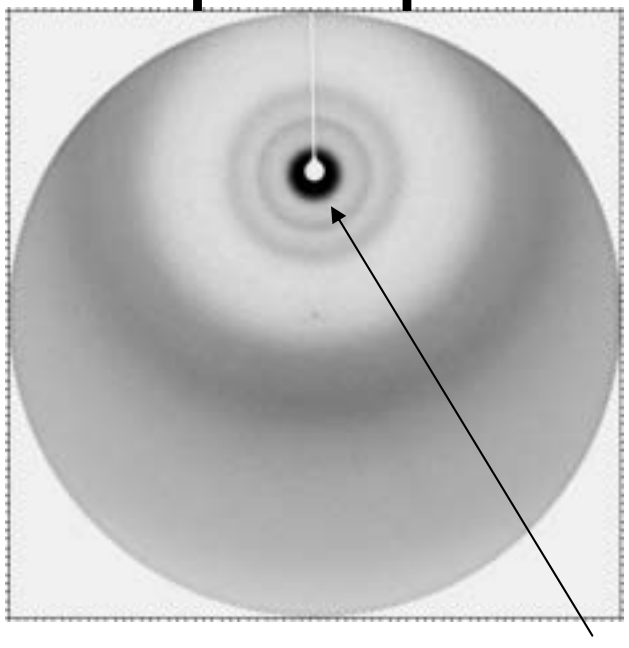

$$\sigma = \sqrt{I_{diff}} / \sin 2\Theta$$

Includes effect of increase in pixels with angle

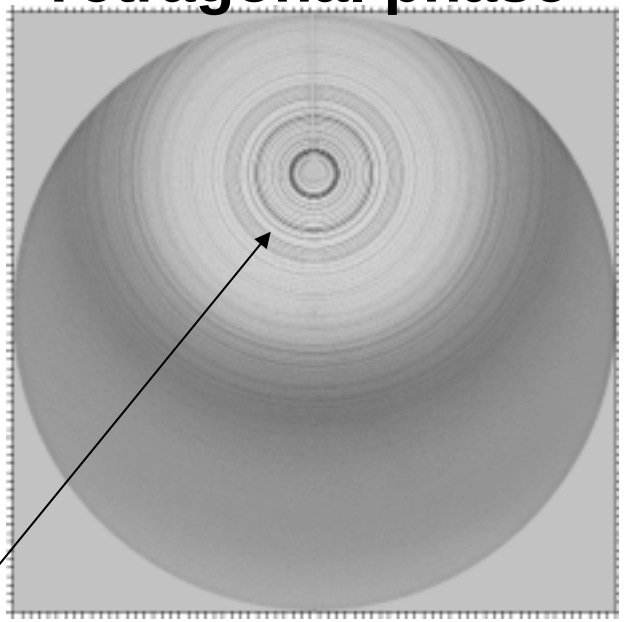


# Lysozyme: 1BM; L=724mm; λ=0.6194Å; 30sec images

**1.5M NaCl/ph5 buffer**
**Amorphous phase**



**4.5hrs later (new sample)**
**Tetragonal phase**



**Small angle scattering & diffuse scattering**

**Do time series experiment & watch crystals form**

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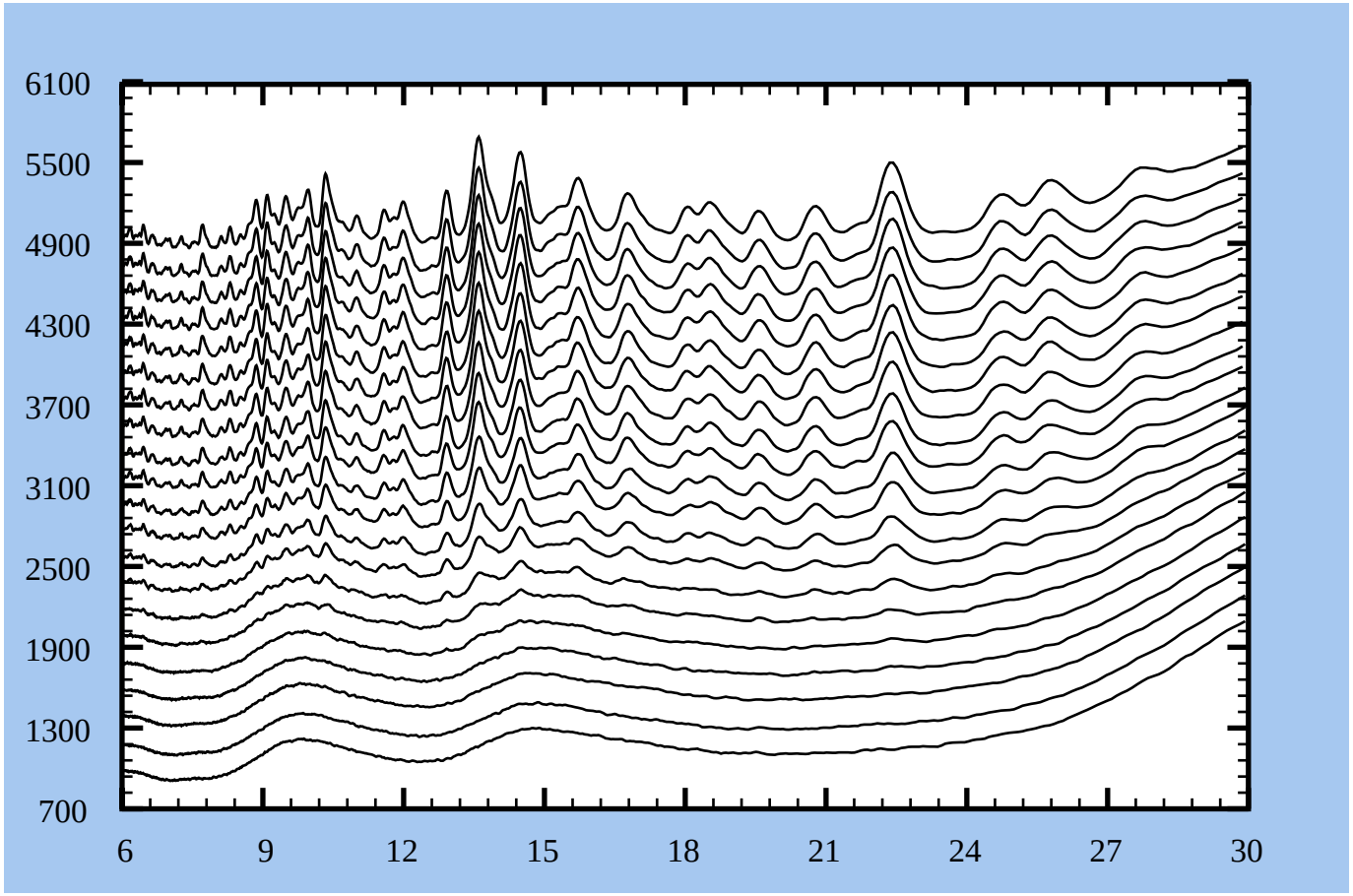
# Protein crystallogenisis from amorphous phase




**Time series:**
**5min steps**
**15min from mixing**
**Results:**
**All growth**
**Vary lattice**
**500Å-1μm xtals**
**Rad. inhibit nucleation**

**11.5mg lysozyme in 200μl 1.5M NaCl/pH6 buffer**

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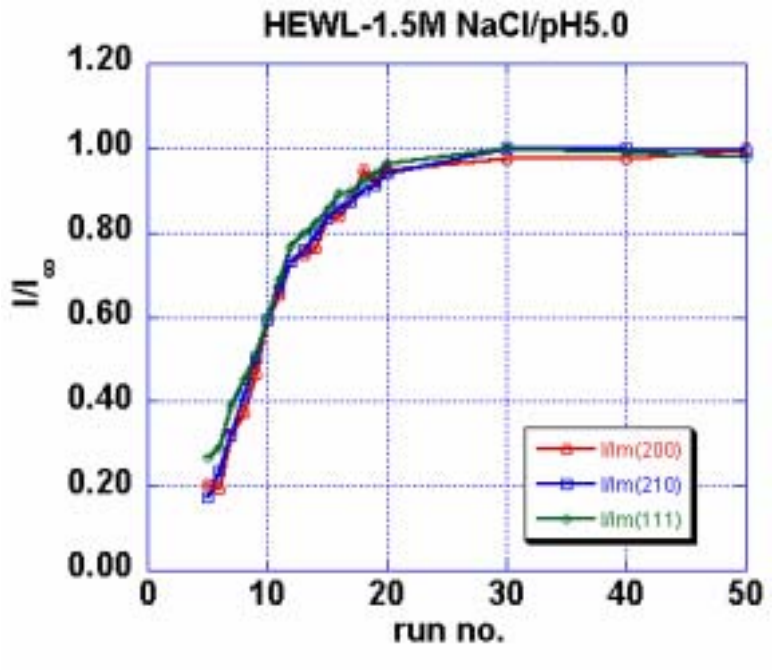
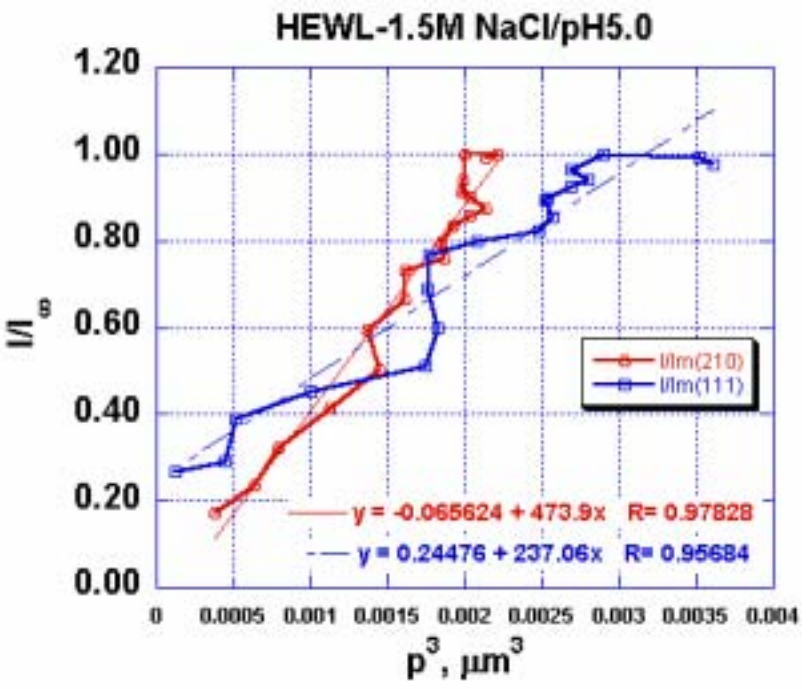
# HEWL crystal growth – intensity vs particle size




**All growth – no nucleation – radiation inhibited**
**Tricky expts.**

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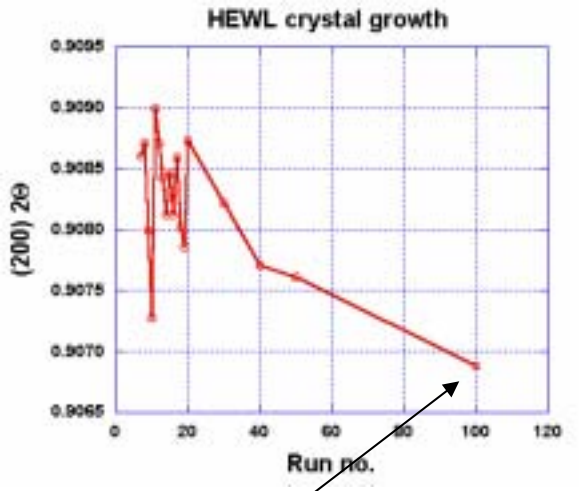
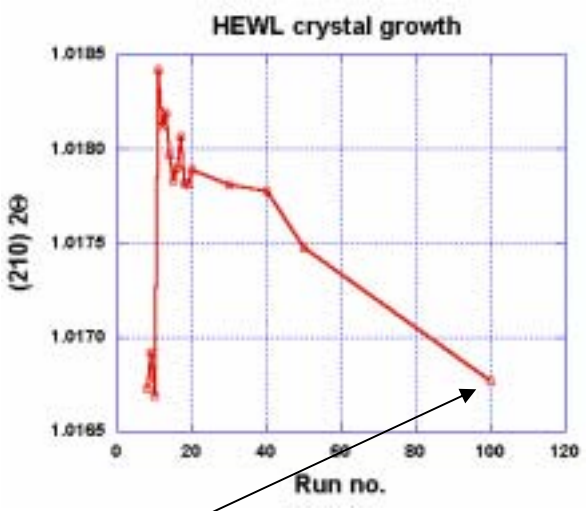
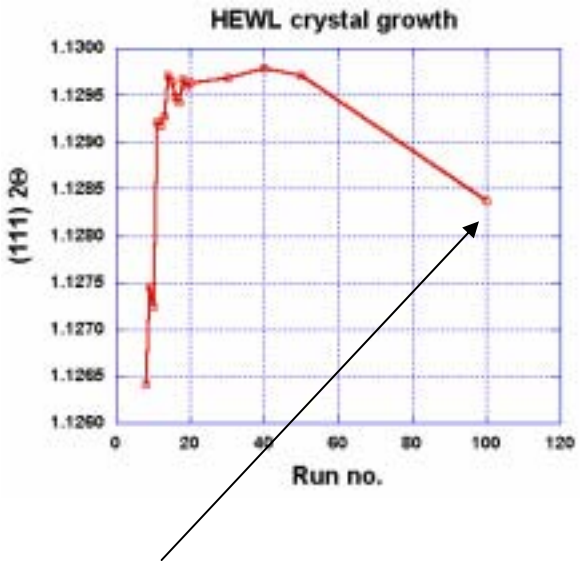
# HEWL crystal growth – surface tension effects?



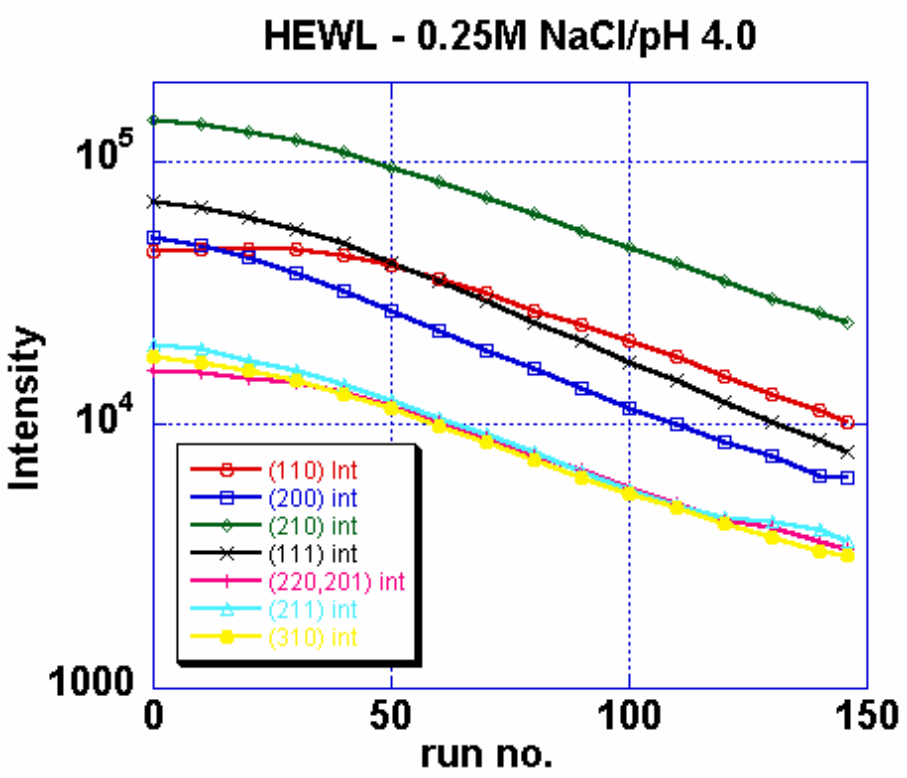

**Last point (Run 100) – fresh sample complete crystallization**

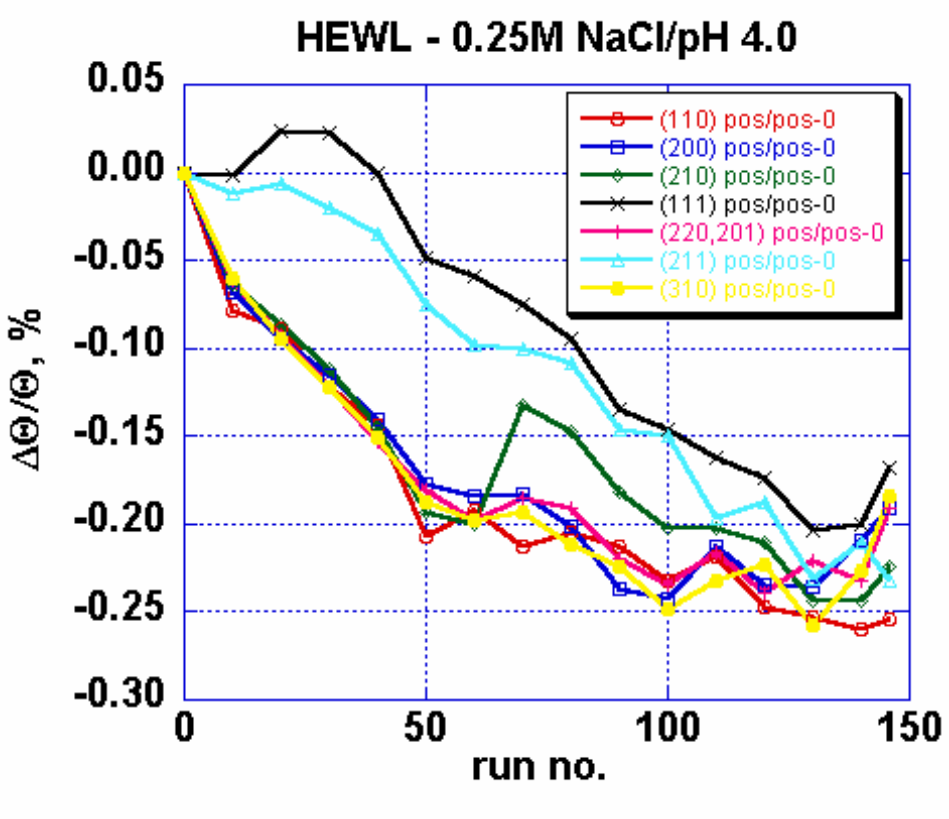
**Grain size 500Å → 0.8μm**

**Lattice decrease first & then slow increase?**

**Surface tension effect? Δa~0.2Å**

# Radiation damage – reflection intensities & positions




**APS 1BM - 30s exposures + 150s delay, room temp.**
**Full sequence wrt NaCl & pH – effects??**
**Immediate changes seen**
**2 stages? - <20min & >20min exposure**

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# Protein polycrystallography – status?

**Fast data collection – image plates; 30s on 1BM;**
**0.035º 2Θ peaks; ~2Å**
**Track crystallization; radiation damage, etc.**
**Wide range of conditions (solvent, temp., etc.) accessible**
**Data analysis – restrained Rietveld refinement**
**Refine from starting models**
**Molecular replacement structure solution**
**“Heavy molecule” solution – complexes**
**1 trial of Xe adsorption – success??**

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# Protein Polycrystallography – future?

**Data collection – higher resolution IP (<100μm vs 300μm);**
**peaks at "best" possible width (~0.01° 2Θ) to <2Å;**
**lower background; <10s exposure with fast readout.**
**Faster *in situ* studies (<10s compared to 150s each step)**
**Refinement techniques – use more SC ideas**
***Ab initio* structure solution – multiple data extraction;**
**heavy atom methods, etc.**

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